1,25-Dihydroxyvitamin D$_2$-26,23-Lactone Interferes in Determination of 1,25-Dihydroxyvitamin D by RIA After Immunoextraction, Bruce W. Hollis (Depts. of Pediatrics, Biochem., and Molecular Biol., Medical Univ. of South Carolina, 171 Ashley Ave., Charleston, SC 29425; fax 803-792-9223)

Numerous assays for the quantification of 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] in serum or plasma have been reported in the past two decades, involving techniques such as isotope dilution–mass fragmentography (1), radioreceptor assay (RRA) (2, 3), and RIA (4). With few exceptions (3), all of these procedures involve exhaustiv e chromatography, including HPLC. The use of HPLC as a purification procedure before assay has been imperative with respect to RIAs for 1,25(OH)$_2$D, because all known antibodies exhibit excessive cross-reactivities to the more abundant metabolites of vitamin D, namely, 25-hydroxyvitamin D [25(OH)D] and 24,25-dihydroxyvitamin D [24,25(OH)$_2$D] (4).

Recently, a new purification format involving selective immunoextraction of 1,25(OH)$_2$D from serum or plasma has been described and used in conjunction with an RIA that incorporates an $^{125}$I tracer. This procedure does not involve previous purification techniques and could represent a significant advance in the detection of circulating 1,25(OH)$_2$D.

We were provided a kit [Gamma-B 1,25(OH)$_2$D] to evaluate by the manufacturer, Immunodiagnostic Systems (IDS; Tyne and Wear, UK). This initial kit performed reasonably well, identifying low circulating 1,25(OH)$_2$D concentrations in patients with chronic renal failure and in vitamin D-deficient rats. However, there were several troubling observations, including abnormally high circulating concentrations of 1,25(OH)$_2$D in healthy subjects and greatly increased concentrations of apparent 1,25(OH)$_2$D in both a vitamin D$_2$-intoxicated subject and a hypoparathyroid patient receiving vitamin D$_3$ therapy. Further, greatly increased apparent 1,25(OH)$_2$D concentrations were observed in several patients with biliary atresia. Because of these initial observations, we obtained another Gamma-B kit and proceeded with a more rigorous investigation to establish a reason for the apparent increased concentrations of 1,25(OH)$_2$D observed previously.

The basis of the Gamma-B kit is selective immunoextraction of 1,25(OH)$_2$D from serum or plasma with a specific monoclonal antibody bound to a solid support. This antibody is directed toward the 1α-hydroxylated A-ring of 1,25(OH)$_2$D (5). To determine the specificity of this extraction technique, we incubated various types of human serum samples with $[{}^3$H]$25$(OH)$_2$D$_3$ or $[{}^3$H]$1,26$(OH)$_2$D$_3$ and determined their recoveries after the Gamma-B immunoextraction procedure. $[{}^3$H]$1,25$(OH)$_2$D$_3$ demonstrated a recovery rate of 78.2% ± 6.0% (x ± SD, n = 6), whereas $[{}^3$H]$25$(OH)$_2$D$_3$ had a recovery rate of only 0.15% ± 0.02% (x ± SD, n = 4). We concluded that the immunoextraction procedure was highly specific for the 1α-hydroxylated forms of vitamin D.

We next compared values obtained for 17 serum samples with the Gamma-B kit with values obtained on the same samples with an established procedure based on solid-phase extraction and purification followed by RRA (3). This selection of samples included six healthy humans, two pregnant humans, two patients with chronic renal failure, one vitamin D$_2$-intoxicated human infant, one vitamin D$_3$-treated hypoparathyroid subject, and one patient with biliary atresia. We also compared four calcium-deficient adult rats. Comparison of the two procedures by regression analysis produced the equation Gamma-B RIA = 1.17 (RRA) + 45.5; r$^2$ = 0.42. Thus, the Gamma-B RIA significantly overestimated circulating 1,25(OH)$_2$D concentrations as compared with the RRA. This overestimation was especially acute with respect to the vitamin D$_2$-intoxicated patient, the hypoparathyroid patient, and the patient with biliary atresia, in whom values were three- to fourfold higher than the respective RRA value.

In an effort to elucidate the reason(s) for overestimation with the RIA, we performed the following study. To normal and chronic renal failure human serum samples we added physiologically occurring concentrations of 1,25(OH)$_2$D$_2$-26,23-lactone (6) or pharmacological concentrations of 25(OH)D$_3$. The samples were then assayed by either RIA or RRA. Results of this experiment are depicted in Fig. 1. Addition of 25(OH)D$_3$ had little effect on the apparent circulating concentration of 1,25(OH)$_2$D as measured by RIA or RRA (Fig. 1A). However, addition of 1,25(OH)$_2$D$_2$-26,23-lactone caused the apparent concentrations of 1,25(OH)$_2$D to increase in a dose–response fashion in both normal and renal-failure subjects when measured by RIA; no such increase was observed when RRA was used (Fig. 1B). With the same normal sample, but not with the renal-failure sample, the RIA continually overestimated the endogenous amount of 1,25(OH)$_2$D (Fig. 1).

It is clear from this study that the IDS RIA overestimates the actual amount of circulating 1,25(OH)$_2$D in all classes of subjects examined except for the renal patients. It is unlikely that this overestimation results from interference involving 25(OH)D, 24,25(OH)$_2$D, or 25,26(OH)$_2$D because the immunoextraction procedure eliminates them. More likely, the overestimation results from other endogenous 1α-hydroxylated metabolites such as 1,25(OH)$_2$D$_2$- 26,23-lactone, 1,24,25(OH)$_3$D, 1,25,26(OH)$_3$D, and (or) calcitriol. It is also possible that conjugated water-soluble metabolites derived from the catabolism of 1,25(OH)$_2$D could interfere in this RIA. The IDS RIA accurately estimates circulating 1,25(OH)$_2$D in chronic renal failure subjects because the majority of these interfering metabolites are derived from renal enzyme systems that degrade 1,25(OH)$_2$D (7, 8). Thus, these catabolic metabolites are not present in the renal patient. The RRA for 1,25(OH)$_2$D is not subject to this overestimation, largely because the other metabolites are removed by chromatographic procedures before assay (3). Also, biologically inactive metabolites such as 1,25(OH)$_2$D$_2$-26,23-lactone and calcitriol are not bound by the vitamin D receptor and thus do not interfere in the RRA (9).

In summary, the Gamma-B kit for determination of circulating 1,25(OH)$_2$D has been poorly characterized. This RIA is not subject to interference by high circulating...
concentrations of 25(OH)D; however, metabolites other than 1,25(OH)2D are being detected, many of which are biologically inactive. The IDS Gamma-B RIA operates under the assumption that 1,25(OH)2D is the only quantitatively significant circulating 1α-hydroxylated vitamin D metabolite, an assumption that is clearly in error. Thus, potential users of this RIA should be cautious in interpreting results derived from the procedure, especially when pathological samples are involved.

References

Lipid Peroxidation Products in Pleural Fluid for Separation of Transudates and Exudates, Abd-El-Rahman M.A. Hammouda,1,4 Magdy M.M. Khalili,2 and Amira Salem3 (Depts. of 1 Biochem., 2 Chest, and 3 Intern. Med., Faculty of Med., Ain Shams Univ., Cairo, Egypt; 4 author for correspondence: fax +202-283-9996)

Classification of pleural fluid as transudate or exudate is used in the management of pleural effusion. A transudate is an ultrafiltrate of the plasma resulting from disturbed dynamics of pleural fluid formation and reabsorption. An exudate results from inflammation or other diseases of the pleura. Measurements of protein concentration and lactate dehydrogenase activity are often used in an attempt to distinguish the two types of effusion (1). Several other indicators have also been introduced to improve the accuracy of diagnosis (2–6). We investigated a new indicator, lipid peroxidation products. Our rationale is that lipid peroxidation is implicated in the inflammatory process (7) believed to be responsible for the exudation of fluid into the pleural space. Lipid peroxidation products already showed a correlation with the acute-phase response in animal-model pleurisy (8).

We retrospectively studied 100 consecutive patients admitted to Ain Shams University Hospitals with established clinical, radiological, and laboratory diagnosis of pleural effusion. These cases included 37 transudates (18 congestive heart failure, 13 liver cirrhosis, and 6 nephrosis) and 63 exudates (36 neoplasms, 14 tuberculosis, and 13 parapneumonia). None of these patients was receiving known antioxidants such as vitamin E and vitamin C. Patients with malignancies had not yet received cytotoxic drugs. All procedures followed were in accordance with the ethical standards for dealing with patients observed by Ain Shams University Hospitals.

The thiobarbituric acid (TBA) assay, in spite of its low specificity, is the most widely used test for measuring lipid peroxidation and is an easy initial screening test. We used a double-heating method according to the modifications suggested for tissue samples by Draper and Hadley (9). To 0.5 mL of the heparinized plasma or pleural fluid in a centrifuge tube we added 2.5 mL of 100 g/L trichloroacetic acid solution and placed the mixture in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000g for 10 min, and 2 mL of the supernate was added to 1 mL of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured at 532 nm. Concentration of TBA-reactive substances was expressed in micromoles of ma-